The Thesis Committee for Ramon Eliseo Cossio
Certifies that this is the approved version of the following Thesis:

The Vascular Effects of a Single Bout of Electronic Cigarette Use

APPROVED BY
SUPERVISING COMMITTEE:

Hirofumi Tanaka, Supervisor

Alexandra Loukas

The Vascular Effects of a Single Bout of Electronic Cigarette Use

by

Ramon Eliseo Cossio

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Abstract

Vascular Effects of a Single Bout of Electronic Cigarette Use

Ramon Eliseo Cossio., M.S. Kin.

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Supervisor: Hirofumi Tanaka

As the use of electronic cigarettes (EC) begins to rise, the need to determine if they are indeed a safe alternative to traditional cigarettes (TC) is of utmost importance. As of 2016, over 15% of US adults age 18 or greater had tried electronic cigarettes. EC are commonly marketed as a safe alternative to TC, but recent studies have observed adverse effects on vascular functions from EC vapors similar to TC. **Aim:** To determine if EC vapor with 5.4% and 0% nicotine by volume have adverse effects on key vascular functions in EC naïve subjects. **Methods:** 16 young apparently healthy subjects found to be free of cardiovascular and respiratory disease were recruited and screened to determine cigarette use. Each subject underwent three separate "vaping" trials with 5.4%, 0%, and sham EC. During each visit, endothelial function (via flow-mediated dilation) and arterial stiffness (via Cardio-Ankle Vascular Index) were measured at baseline, immediately post protocol, 1 hour post protocol (1hr), and 2 hours post protocol (2hr). A repeated measures ANOVA was used to determine if there were any significant time versus group interactions. **Results:** There were no significant changes in flow-mediated dilation (FMD), brachial blood

pressure, and cardio ankle vascular index (CAVI) Score throughout the experiments lasting 2 hours. **Conclusion:** Vaping electronic cigarettes regardless of nicotine content are not significantly different from each other and do not produce lasting effects over the course of a 2-hour trial.

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Review of Literature

Electronic Cigarette: Background

Electronic cigarette (EC) use increased 900% among high school students in a period of four years. (HHS 2016) EC are marketed as safe alternative to tobacco cigarette TC but little research has been conducted on the chronic or acute effects on the cardiovascular system when exposed to EC vapor. To date, several chromatography studies have attempted to determine the overall aerosol content of various electronic cigarette products with varied levels of aldehydes, carbonyls, metals, and volatile organic compounds. (Goniewicz et al. 2013, Cheng 2014, Huang et al. 2018, Javed et al. 2017) Additionally, human and animal respiratory studies have found evidence of (EC) vapor as detrimental to lung function in the form of increased inflammation and irritation of the bronchial tubes.

There is a larger gap in the literature when determining the effects of EC vapor upon the human vasculature. It is well established that TC smoke causes excess inflammation within the endothelium of human arteries, which in turn causes endothelial dysfunction that leads to atherosclerosis. (Ambrose and Barua 2004) Thus, the research used to determine TC effects is uniquely suited to determine the effect of EC on cardiovascular function as the modes of delivery are similar. At the time of this writing, there had been only two human studies and one animal study that have studied the effect of EC on vascular function, inflammation, blood pressure, and pulse wave velocity. Carnevale et al. (2016) reported that the effects of EC and TC were not significantly

different in their effects on endothelial function as determined by flow-mediated dilation (FMD), but found a significantly reduced effect of EC on inflammatory biomarkers. In terms of acute effects upon arterial stiffness, Vlachopoulos et al. (2016) found that EC was significantly less detrimental on pulse wave velocity (PWV) after five minutes when compared with TC. However, when the time of vaping was extended to thirty minutes, EC was found to be similar to the detrimental effects of TC. (Vlachopoulos et al. 2016) In a striking study conducted by Olfert et al. (2017), mice exposed to EC vapor for a total of four hours per day, five days a week, for a period of eight months observed a threefold increase in PWV of the mice exposed to EC vapor when compared with those exposed to air.

While studies continue to show a close similarity to the effects of EC vapor to TC smoke, what is yet to be determined are the effects of EC vapor void of nicotine. It is well accepted that many of the detrimental effects caused by TC are a result of toxic substances released when combustion of TC occurs. While EC do not burn, they still rely on the heating of constituents, which have been observed to produce potentially harmful substances. (Glasser et al. 2017) Goniewicz et al. (2014) studied 12 brands of electronic cigarettes and their produced aerosols and reported that all contained some amounts of aldehydes, volatile organic compounds, tobacco-specific nitrosamines, or metals. These substances have been linked to be the main cause of oxidative stress in TC. (Benowitz and Burbank 2016)

Endothelial Function

A strong indicator of emerging cardiovascular disease is vascular dysfunction in the form of reduced vasodilatory responses to physiological and/or pharmacological stimuli. (Endemann and Schiffrin 2004) In response to increased metabolic demands exerted upon the vasculature, the vasodilatory molecule nitric oxide (NO) is produced as a result of the increase in endothelial nitric oxide synthase activity. (Endemann and Schiffrin 2004) NO is responsible for directly acting upon the vascular smooth muscle as a vasodilator, reducing platelet coagulation, and decreasing inflammation. (Endemann and Schiffrin 2004) In contrast, increases in vascular inflammation give rise to the reactive oxygen species (ROS) peroxynitrite, which increase low density lipid oxidation and further scavenges eNOS. C-reactive protein is also increased in a proinflammatory state resulting in the endothelial dysfunction.

Endothelial function can be used as an early prognostic tool for future cardiovascular disease and as such can be noninvasively measured utilizing flow FMD. (Ras et al. 2012, 2013) The hyperemia occurring after occlusion causes shear stress upon the arterial wall stimulating the release of endothelial nitric oxide synthase(eNOS) and further catalyzing NOS, which in turn vasodilates the vessels through relaxation of the smooth muscle cells. (Ras et al. 2012, 2013) FMD is performed by occluding forearm blood flow beginning at the antecubital fossa. An ultrasound probe is used to measure a cross section and record the brachial artery 6-10 cm above the occluded site. After a five-minute occlusion period the cuff is released, and measurement is commonly recorded for several minutes to determine peak blood flow and vascular dilation. (Ras et al. 2012, 2013) If

endothelial dysfunction is present, little to no change will be observed due to the decreased availability of eNOS.

TC and EC exposure has been shown to negatively and acutely affect endothelial function. (Carnevale et al. 2016, HHS 2011) Dysfunctional endothelial cells induced via TC have an increased expression of adhesion molecules leading to vascular inflammation, are unable to resist thrombosis, and result in reduced nitric oxide bioavailability. (HHS 2011) TC smoke is known to have an abundance of free radicals such as superoxide, which reduce NO availability further increasing vascular oxidative stress. (Benowitz and Burbank 2016) These effects are short lived in healthy non-smokers, but repeated exposure leads to arterial stiffening, which further reduces endothelial function as calcification of the endothelial wall prevents sheer stress from stimulating eNOS. (HHS 2011)

Arterial Stiffness

Arterial stiffness is the physical stiffening of vascular structure mediated by arteriosclerosis. (Tölle et al. 2015) Arteriosclerosis causes calcification and remodeling of the medial or endothelial lining of the arterial wall. Traditionally, arterial stiffness has been measured utilizing the reference standard measure of aortic PWV. However, it is reliant on blood pressure at time of measurement, which may confound results attempting to determine the effects of pharmacological agents. (Shirai et al. 2011) Thus, the Cardio Ankle Vascular Index (CAVI) has been a novel development in the measurement of arterial stiffness as demonstrated by Kubozono et al. (2007) CAVI is measured from the aorta to the tibial artery located at the ankle and utilizes the diastolic and systolic blood pressures

measured at the brachial artery. (Shirai et al. 2011) CAVI can be summarized by the following equation; CAVI = a [(2ϱ/ΔP) × ln(Ps/Pd) PWV^2] + b

Much like endothelial function, TC have been linked to arterial stiffness mediated by increased states of inflammation, platelet aggregation, and decreased eNOS expression. (Ambrose and Barua 2004) Stiffness of the vasculature in turn results in increased BP and stress upon the heart. Thus, smokers are at increased risk for cardiovascular diseases and early mortality. (HHS 2011)

Nicotine

The substance nicotine is and has been of profound interest in all research concerning tobacco products, as it is the main component linked to the addiction of said products. (Luttrell et al. 2014) Nicotine delivered via inhalation is transported to the lungs, where it is then absorbed into the blood stream, and continues on to the brain where it binds to nicotine acetyl cholinergic receptors (nAChRs) within 15-20 seconds (Benowitz and Burbank 2016, Benowitz 2010) nACHRs stimulate the release of dopamine resulting in reinforcement of a pleasurable experience. (Benowitz 2010, Dajas-Bailador and Wonnacott 2004) The half-life of nicotine in itself is two hours, owing to the repeated need to smoke. Typically, smokers spend 6-8 hours of the waking day delivering nicotine to the brain and stimulating nACHRs. Thus, nicotine addiction may result in repeated use of tobacco products, which in turn causes repeated exposures to harmful substances as repeated stimulation of nACHRs result in a blunting effect requiring larger doses of nicotine. (Benowitz and Burbank 2016)

Additionally, nicotine may be responsible for acute effects on the human cardiovascular system through sympathetic nervous system stimulation and release of catecholamines. The increase in norepinephrine and epinephrine stimulated by β1 receptor cause a rise in cardiac output by increasing heart rate and contractility. While stimulation of the α receptor results in peripheral vasoconstriction thereby increasing blood pressure by up to 5-10mmHg and cardiac filling (Benowitz and Burbank 2016, HHS 2010, Cryer et al. 1976) Paradoxically, coronary blood flow has been shown to increase in healthy subjects primarily due to the increase in cardiac output, which in turn stimulates flow-mediated vasodilation within the coronary artery. (Benowitz and Burbank 2016, Czernin and Waldherr 2003) As cigarette use increases over a lifetime, blunting of this phenomenon can be observed in smokers who have been diagnosed with cardiovascular disease. (Cryer et al. 1976, HHS 2010)

Still nicotine itself has not been linked as the primary mediator of cardiovascular disease in smoking. In fact, studies conducted on Swedish snuff (snus) did not see an association with myocardial infarction despite delivering the same level of nicotine concentrations as a TC. There is some evidence to suggest that a constant beta adrenergic and sympathetic stimulus may result in cardiac remolding and hypertension. (van Berlo et al. 2013, Jensen et al. 2012) However, determining nicotine's role in cardiovascular disease is difficult to determine as many of the vehicles used to deliver nicotine are detrimental to health themselves.

Menthol

Menthol's role in TC is still relatively inconclusive with multiple studies observing an enhancement of inflammatory effect or no difference in effects when compared with a mainstream TC. (Lin et al. 2017, Gaworski et al. 1997, Pickworht et al. 2002, Abobo et al. 2012) Of note, Fetterman et al. (2018) observed cell death, increased expression of Interluken-6, and impaired nitric oxide production when studying the effects of menthol independent TC. These findings were in agreement with an earlier study conducted by Leigh et al. (2016).

Topical and oral studies on menthol have determined a slew of beneficial properties as an anti-inflammatory and vasodilatory agent. Johnson et al. (2009) found menthol to be a vasodilatory agent in human cutaneous vessels involving the possible stimulation of nitric oxide pathways and transient receptor potential melastatin 8 (TRPM8) when observed *In Vivo*. Xiong et al. (2017) found oral ingestion of menthol attenuated cold induced hypertension via the stimulation TRPM8, menthol was linked to reductions in reactive oxygen species and vasoconstriction.

Propylene Glycol (PG) and Glycerol (VG)

PG and VG are the main constituents of EC liquid and both have been found to be safe for human consumption via the FDA. However, these properties when heated have not been widely studied. PG mist and aerosol have been found to be irritants to human respiratory and ocular function when used as theatrical smoke and in firefighter training. (Varughese et al. 2005, Wieslander et al. 2001) The question as to whether PG and VG are

safe for human use is not limited to aerosols as is evident in their historical use in foods and medicine. As vehicle for intravenous delivery, PG has been linked to sever side effects such as arrhythmias and seizures. (Arulanantham and Genel 1978, Martin and Finberg., 1970)

More recently, researchers have found shisha pen vapor, whose liquid was composed of PG and VG, sufficient to cause airway irritation. (Kienhuis et al. 2015) While Goel et al. (2015) determined that there were significant reactive oxygen species created via heating of EC solvents PG and VG. Of concern are poor manufacturing techniques resulting in the production ethyl and propylene glycol mix being packaged into e-liquids. (Longo et al. 2016) Herrington and Meyers (2015) reported up to 70 unidentified compounds through chromatography studies in four of the most popular brands of EC. Those compounds included formaldehyde, acrolein, acetone, and propylene oxide. Finally, this year's review by the National Academy of Sciences, Engineering, and Medicine has stated that decomposition of EC solvents leads to the formation of carbonyls such as formaldehyde and acrolein. (Daynard et al. 2018)

Summary

ECs and their aerosol constituents are now being heavily studied around the world. It has been confirmed by previous studies that EC with nicotine have effects on PWV, BP, and endothelial function. However, it is not clear whether these effects are direct results of nicotine or indirect effects of significant amount of potentially harmful substances being inhaled that can and will lead to development of CVD through chronic use. It is therefore

necessary to explore nicotine free ECs to greater extent in order to determine if they are truly safer than TCs.

Introduction

The use of electronic cigarettes has risen drastically over the last few years and is of particular concern because young adults are especially drawn to their use (HHS 2016, Regan et al. 2013). Traditional cigarettes are well studied and are known to cause significant harm to the cardiovascular system. Just one traditional cigarette can increase arterial stiffness within minutes of smoking and chronic use may lead to arteriosclerosis, which in turn may lead to the development of more sever cardiovascular diseases. (Kubozono et al. 2011) Currently, very little is known about the effect of electronic cigarettes in general and on cardiovascular function in particular. Electronic cigarettes are fruity favorable mild vapors that pose little irritation and therefore are more enjoyable of a habit (HHS 2016, Grana et al. 2014). Accordingly, electrotonic cigarettes are perceived to be much less harmful. However, recent letter to the editor has described adverse effects caused by electronic cigarettes in regard to arterial stiffness in chronic cigarette smokers (Vlachopouls et al. 2016). Additionally, endothelial function decreased significantly when the subjects were exposed to electronic cigarettes (Carnevale et al. 2016). More recently, Olfert et al. (2017) found significant increases in arterial stiffness in mice when they were chronically exposed to EC vapor for five days a week over eight months.

A question that has not been explored is whether EC vapor void of nicotine have any adverse effects. Chromatography studies have found a variety of chemicals including carbonyls, aldehydes, and trace metals from the super-heating of PG and VG (Goniewicz et al. 2014, Glasser et al. 2017, Cheng 2014). In the case of TC smoke, the volatile compounds from combustion, such as tar and formaldehyde, are the main antagonist to vascular disease (Ambrose and Barua 2004). Currently, it is unknown if non-nicotine vapor has any impacts on vascular function.

Therefore, the primary purpose of the present study was to assess the acute changes in arterial stiffness and endothelial function after smoking EC for a vaping period of six minutes. To maximize the effects of EC and to minimize the downregulation associated with chronic exposure, we studied subjects who are naïve to EC and TC. Utilizing three conditions; an EC with 5.4% nicotine by volume, an EC with 0% nicotine by volume, and Sham menthol inhaler without vapor or nicotine. We hypothesized that both vaping conditions would cause significant changes to arterial stiffness and endothelial function when compared with a sham menthol inhaler.

Methods

Subjects and Procedures

A total of 16 young apparently healthy adults were recruited via convenience sample at the University of Texas and the surrounding Austin, Texas area. All subjects were between the ages of 20-30 years and were asked to complete a health history questionnaire to determine their eligibility. Volunteers with overt cardiovascular and pulmonary diseases (hypertension, diabetes, asthma) and those who had regularly used tobacco and nicotine products within the last 6 months and who were taking cardiovascular acting drugs were excluded. Additionally, female participants were tested only during the follicular phase of their menstrual cycles to minimize the confounding hormonal effects.

Subjects reported to the laboratory having fasted for a minimum of 8 hours and having abstained from vigorous activity, caffeine, alcohol for a minimum of 12 hours. Upon the subjects' arrival at the Cardiovascular Aging Research Laboratory, their height and body weight were recorded followed by a supine resting period of ten minutes in a dimly lit temperature-controlled room. At the completion of the rest periods, a baseline measurement of arterial stiffness and endothelial function were taken. Upon completion of baseline assessments, subjects were escorted to a previously selected outdoor area and asked to vape an electronic cigarette, which was a combination of a battery (Cirrus 3) and cartridge (Menthol Flavor Clear Draw Max 5.4% or 0%) or a sham cigarette (Harmless Cigarette Quit Smoking Aid). EC containing 5.4% nicotine by volume (NBV) was selected as it is considered a higher strength vapor, that would likely produce a robust response. Doses of 16 mg and .8mg or about 1.6% and <1% NBV respectively have been used in previous studies. (Olfert et al. 2016, Crippa et al. 2018). The vaping protocol was a total of six minutes in length, consisting of four second inhalations every twenty seconds culminating into eighteen puffs in total. The researcher verbally instructed all subjects

when to inhale and exhale, with an emphasis on drawing vapor into the lungs. The order of ECs was randomized.

Subjects were then asked to return to the laboratory for an acute post EC assessment. Assessments were repeated post one hour and post two hours to determine time (i.e., residual) effect on the vasculature. Subjects completed this protocol a total of three times nonconsecutively, with a minimum of forty-eight hours between testing days. All measures were recorded by a single researcher.

Cardioankle Vascular Index (CAVI)

All measurements were taken with a computerized semi-automatic noninvasive body pressure monitoring device VaSera (Fakuda Denshi, Bunkyo-ku, Tokyo, Japan). CAVI is a measure of arterial stiffness and was calculated from pulse wave distance divided by transit time. Blood pressure cuffs placed upon ankles and arms were inflated to a pressure of 30-50 mmHg in order to record pulse waves but not affect hemodynamics (Shirai et al. 2006). Pulse wave velocity was measured by summing closure of the aortic valve and brachial notch appearance upon the wave form, plus the time between the rise of the brachial and ankle pulse wave. The CAVI score above a 9 indicates possible arteriosclerosis, and CAVI scores below 8 signify no measurable arterial stiffening. (Shirai et al. 2011, Saiki et al. 2016).

Flow-Mediated Dilation

Flow-mediated dilation (FMD) is a noninvasive method to assess vascular endothelium dependent vasodilation by measuring the brachial artery's responsiveness to ischemic stress. By use of an automated diagnostic ultrasound system (UNEXEF-38G, UNEX corporation, Sakae Naka-ku, Nagoya, Japan). The system is equipped with an

electric linear array transducer operating at 10 MHz. While subjects rest in supine position, a blood pressure cuff was placed on the forearm with the proximal edge of the cuff above the subject's antecubital fossa and second blood pressure cuff was placed on the contralateral arm for standard blood pressure measurements (Tomiyama et al. 2015). Cross-sectional images of the artery were acquired utilizing the automated probe, which self-adjusted to provide a clear longitudinal image of the artery and begin baseline measurements (Tomiyama et al. 2017). After the acquisition of baseline measurement, the cuff inflated to 50 mmHg above resting systolic blood pressure for 5 minutes. Upon cuff deflation, ultrasound-derived measurements of the brachial artery diameters and blood flow velocity were taken for 2 minutes. FMD was calculated by the following equation: (maximum diameter – baseline diameter) / baseline diameter x 100 (Tomiyama et al. 2015).

Statistical Analyses

Results were analyzed IBM SPSS statistics version 24, utilizing a repeated measures ANOVA for all variables comparing time and group effects. All p-values reported utilizing Greenhouse-Geisser correction with any significant effects further analyzed utilizing pairwise comparisons.

Results

Selected subject characteristics are presented in Table 1. Subjects were young healthy and had normal body weight. (**Table 3**). CAVI scores tended to increase during the post measurement period but there was not a significant group x time interaction (p=.31) (**Figure 1** and **Table 2**). FMD tended to decrease slightly post vaping in all conditions but results were not found to be statistically significant (**Figure 2** and **Table 2**). Both systolic and diastolic BP tended to increase post vaping but was not significant (**Figures 3** and **4** and **Table 2**).

Discussion

In the present study, our working hypothesis was not supported by the results as no significant changes in subclinical vascular functions were observed with the ECs containing 5.4% and 0% nicotine. Even though we observe slight changes in all variables, including CAVI, systolic and diastolic BP increasing immediately post a vape and FMD declining, these changes were modest at best and did not reach statistical significance. Interestingly, the sham condition displayed the same pattern of increase post vape as other ECs containing nicotine. We believe that this may be attributed to our novel method of sham control involving a menthol inhaler and the use of blinded EC naïve subject. In other studies, the sham control was the use of an expended or empty EC device, which can be recognized easily by th participants (Carnevale et al. 2016, Vlachopoulos et al. 2016, Crippa. 2018).

CAVI measurements used in the present study are well established in detecting arterial stiffening and are relatively non-technical in nature. Unlike carotid-femoral pulse wave velocity (PWV), the placement of femoral and carotid tonometers is not necessary, and measurements are less tedious, accounting for less researcher bias. TC studies utilizing both brachial-ankle PWV and CAVI have shown strong correlations between the methods, and both measures increased significantly after tobacco smoking (Kubozono et al. 2011). Given this, we could compare our use of CAVI to Vlachopouls et al. (2016) who used PWV. Although they observed a significant increase in PWV after five minutes of vaping, it is a letter to the editor and has very limited information to interpret the data. More recently, Franzen and colleagues (2018) observed elevated PWV but did not report

significant changes. We believe that our lack of significance may be found in the chosen statistical analysis determining the group x time interaction rather than significant changes from baseline measurement to post measurement.

We did not observe significant difference between conditions when it came to FMD change likely caused by an inappropriate does of nicotine. While we did not use a TC for comparison as others studies have, we did observe no significant differences in FMD between the 5.4% and 0% conditions. This begs to question if a 0% EC would display similar changes in comparison to a TC without nicotine. Of noted strength to our form of measurement is the use of the new UNEX Semi-Automated FMD device, as it is the device that selects the appropriate image for measurement, has an automated occlusion control, and records post occlusion automatically. Unlike the common form of measure ($\geq$200mmHg), the one used by our occlusion pressure was 50 mmHg above the baseline SBP measure, likely attributing to lower % change (Harris et al. 2010). Like CAVI, all measures were taken by one researcher and analysis was automatically generated real time, allowing little room for introducing investigator bias or variability.

Visible increases in SBP and DBP were observed in the 5.4% EC condition but only DBP was found to have a time x group significance, further analysis and review of pairwise comparisons resulted in no significant results. This is in contrast to a recent study by Crippa et al. (2018) who found significant changes in both SBP (9.6±4 mmHg) and DBP (7.1±3.9 mmHg) when comparing with placebo trials in subjects with mild hypertension. Lack of hemodynamic responses to the high strength nicotine product used in the present study may suggest inadequate dosing for subjects who were naïve to EC or

tobacco smoking. However, a few subjects experienced sever irritation during the bout of 5.4% NBV vapor. This may be a cause for concern as EC users tend to vape more regularly and draw in longer puffs, with experienced users inhaling for as long as eight seconds, double the time of our current protocol (Olfert et al. 2018).

Another strength of this study is the use of young 20-30 year old subjects, who were healthy, active, and the majority naïve to both EC and tobacco smoking. We believe this may be a unique sample demonstrating the controversial cardioprotective qualities of frequent exercise. A study by Park et al. (2014) observed no significant differences in PWV between active smokers and active non-smokers leading the researchers to conclude that regular exercise may be protective against the negative effects of TC. Many of the previously mentioned studies utilized current or former smokers (Crippa et al. 2018, Franzen et al. 2018, Vlachopouls et al. 2016). It seems necessary to classify subjects in terms of regular physical activity in future studies to rule out if any detrimental effects exist for the active population of EC users. The use of a familiarization trial was implemented to ensure proper inhalation of the EC product. As mentioned, the use of smokers and smaller levels of NBV in previous studies likely resulted in better inhalation of vapor product, which we were not able to replicate.

Limitations of this study were evident in use of non-consecutive trials which lasted just under 3 hours. As required by the protocol and minimum of 48 hours was given between trials, often leading to scheduling conflicts. Thus, a number of subjects finished their trials over an extended period of time. Another important limitation is a small sample size. Lastly, we were limited in our vaping location, and the protocol required subjects to

walk close to 50 m to and from the vaping location. Future studies should make every effort to reduce movement of subject and if possible test and measure subject in the same location.

The safety of electronic cigarettes remains in question, especially with a multitude of vapor liquid and device options available in the market. This study has determined that electronic cigarettes regardless of nicotine presence are not significantly different from one another as evident by the similar trend in responses regardless of condition. Future studies would benefit in comparing non-nicotine vapors at various heating temperatures as a study using the chromatography has observed changes in propylene glycol and glycerol under greater temperatures.

Tables and Figures

Table 1. Selected Subject Characteristics

Variable	n or Mean ± S.D.
Participants (n)	16
Sex (n)	M (9) F (7)
Age (yr)	24±2.5
Height (m)	1.73 ± 0.08
Body weight (kg)	69.5 ± 10.3
BMI (kg/m^2)	23.2 ± 2.8
SBP	117 ± 8
DBP	68 ± 4
Heart Rate(bpm)	54 ± 8

BMI= Body Mass index, SBP=Systolic Blood Pressure, DBP=Diastolic Blood Pressure, bpm=beats per minute

Table 2. Changes in vascular and hemodynamic measures during the experimental periods

Variable	Group	Mean Baseline	S.D.	Mean Post	S.D.	Mean +1hr	S.D.	Mean +2hr	S.D.
CAVI	5.4%	5.82	0.69	6.23	0.76	6.04	0.88	5.90	0.75
	0%	5.91	0.59	6.04	0.74	6.03	0.53	6.11	0.66
	Sham	5.70	0.64	5.93	0.89	5.95	0.81	5.98	0.78
FMD	5.4%	5.63	1.75	5.23	1.68	6.12	2.14	5.56	2.57
	0%	5.73	2.82	4.91	2.03	4.98	2.21	5.24	2.49
	Sham	6.64	2.45	5.60	2.40	6.64	2.01	6.16	3.24
SBP	5.4%	119	9.64	124	9.46	121	9.56	121	9.03
	0%	115	7.65	118	9.64	120	7.84	119	9.58
	Sham	117	6.24	119	7.46	120	7.25	120	7.07
DBP	5.4%	69	4.34	73	4.97	71	6.15	70	5.40
	0%	66	4.09	68	4.71	70	5.09	68	4.67
	Sham	68	3.42	68	5.64	71	6.05	69	5.13

CAVI = cardio ankle vascular index, FMD = Flow-mediated dilation, SBP = systolic blood pressure, DBP = diastolic blood pressure, 5.4% = Nicotine by volume, 0% = Nicotine by volume, Sham = Control

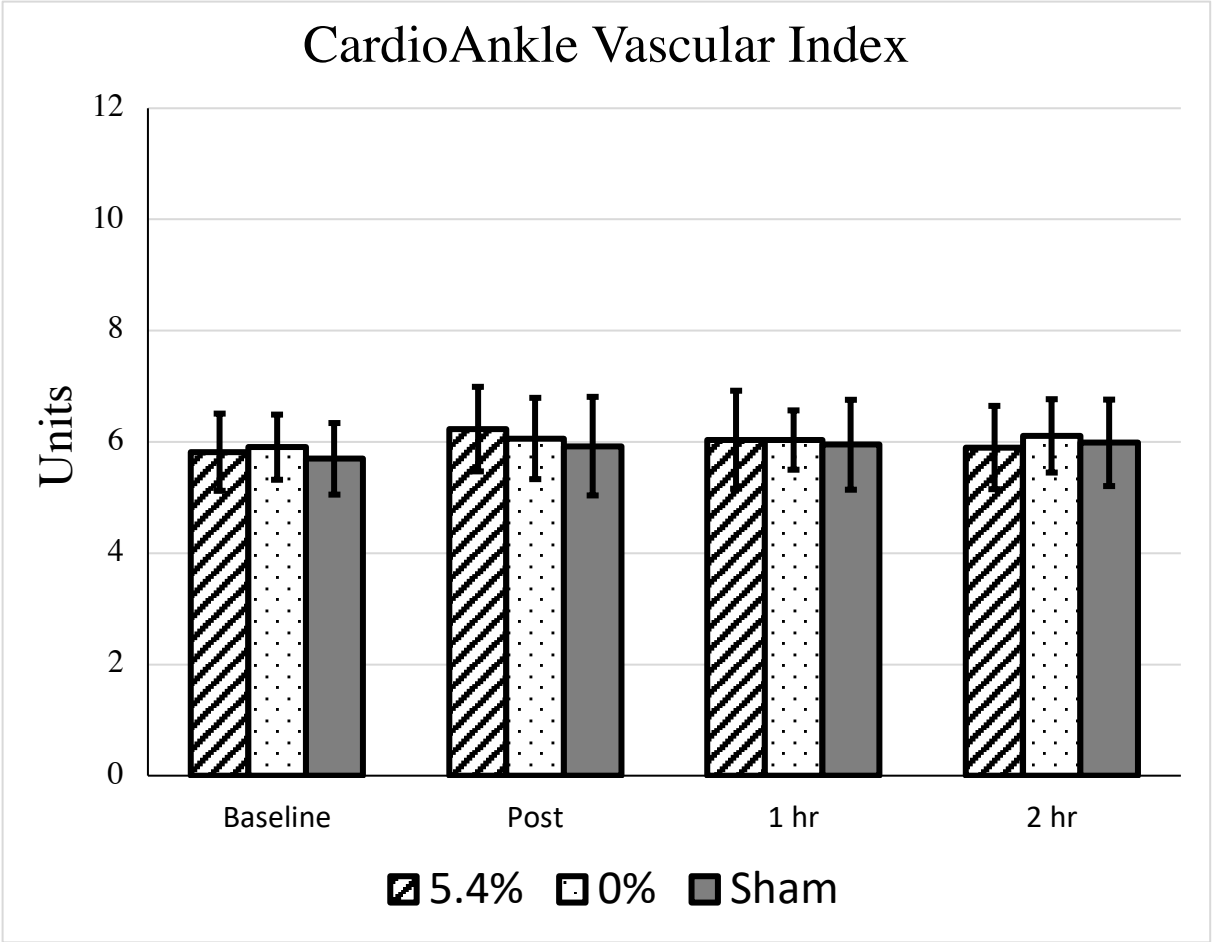

Figure 1. Mean±S.D. Cardio Ankle Vascular Index (CAVI) scores at baseline, immediately post, at 1 hr, and at 2 hr post EC exposure

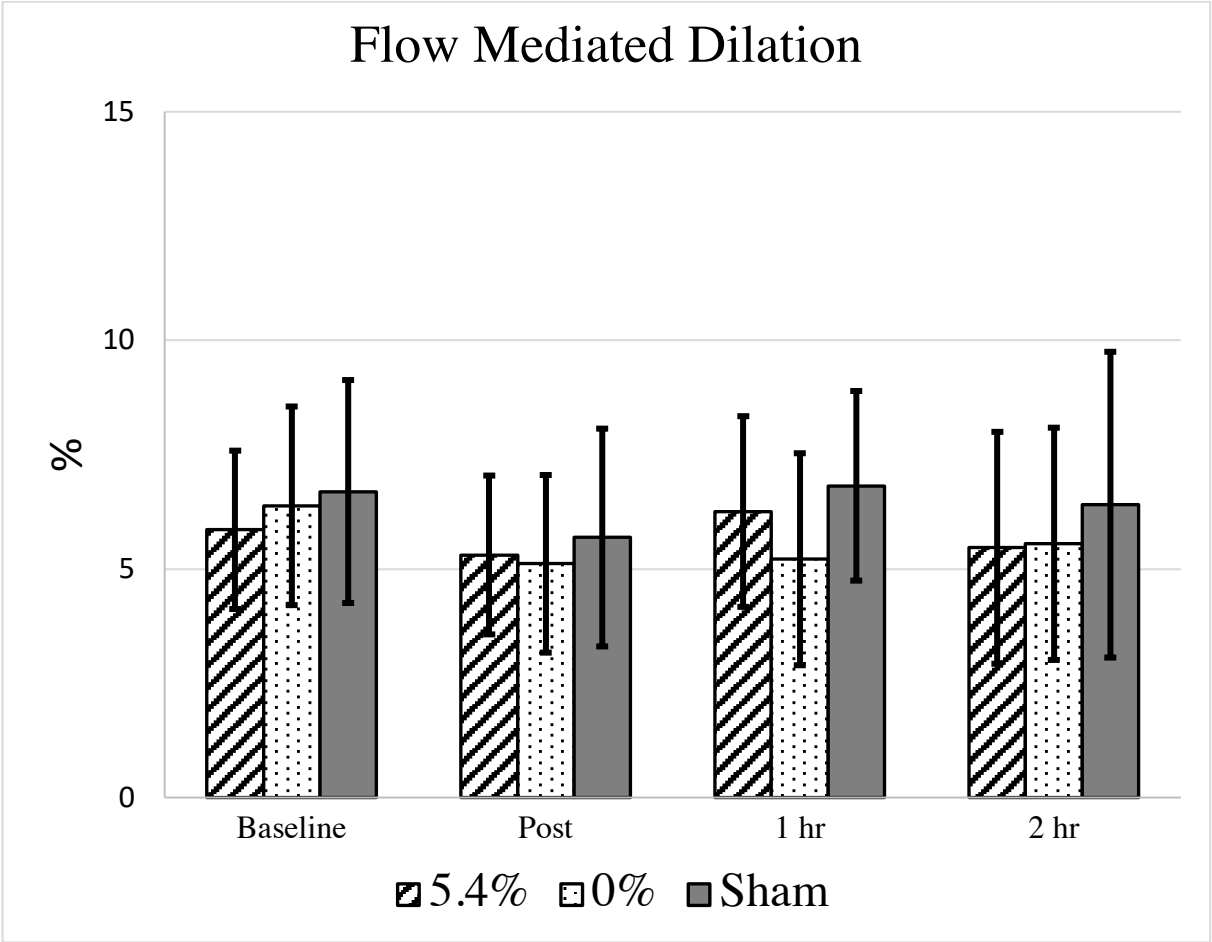

Figure 2. Mean±S.D. flow-mediated dilation at baseline, immediately post, at 1 hr, and at 2 hr post EC exposure

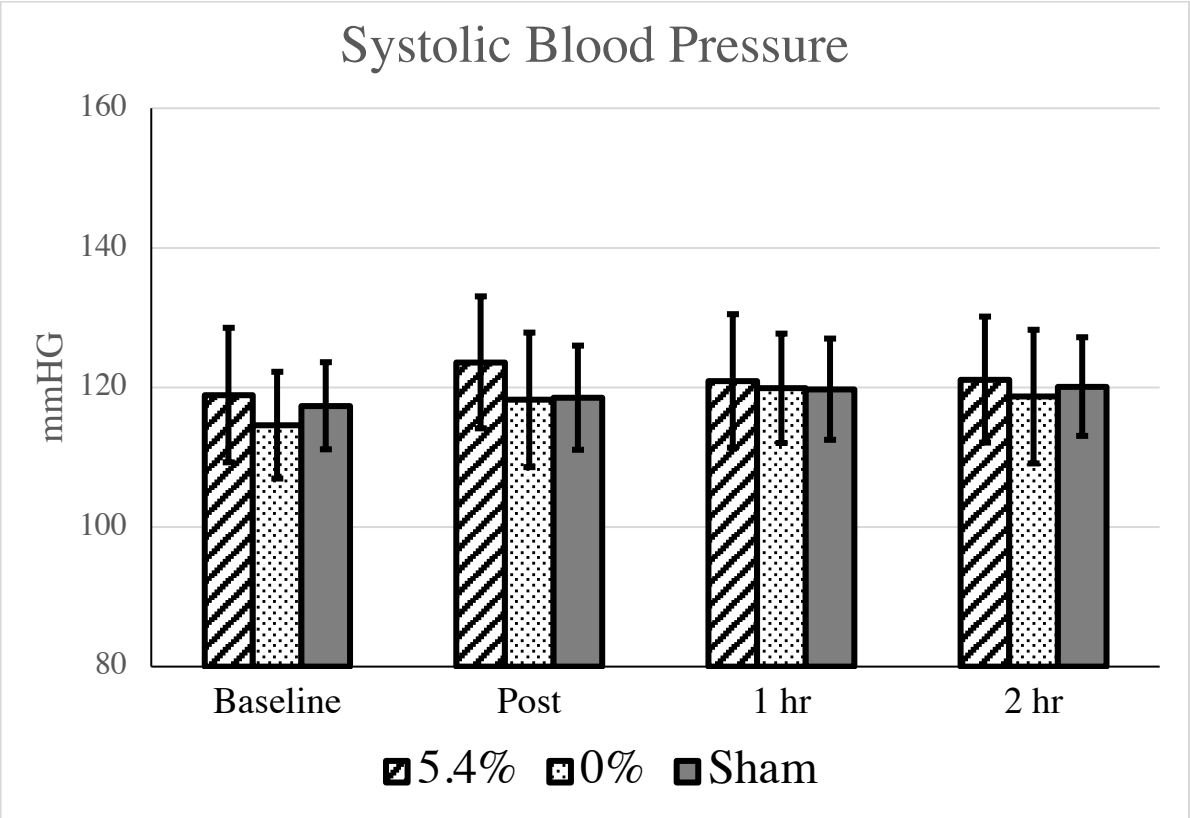

Figure 3. Mean±S.D. brachial systolic blood pressure at baseline, immediately post, at 1 hr, and at 2 hr post EC exposure

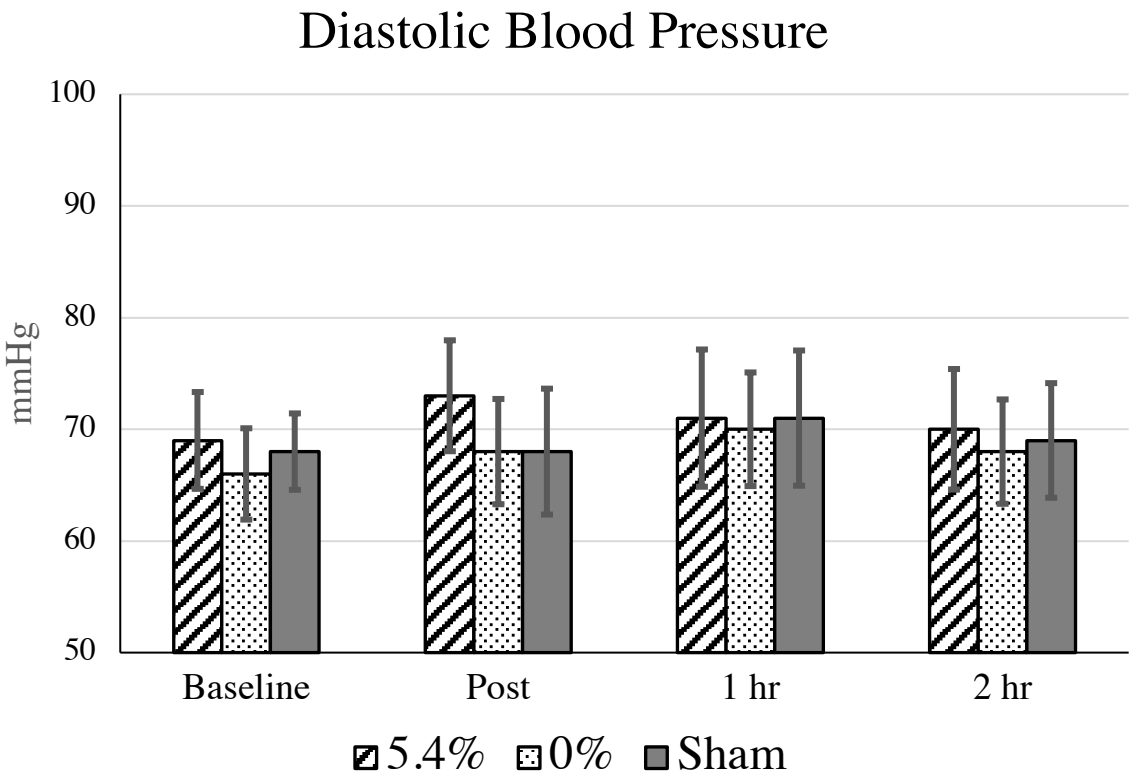

Figure 4. Mean±S.D. brachial diastolic blood pressure at baseline, immediately post, at 1 hr, and at 2 hr post EC exposure

References

1. Abobo, C., Ma, J., & Liang, D. (2012). Effect of menthol on nicotine pharmacokinetics in rats after cigarette smoke inhalation. *Nicotine & Tobacco Research, 14*(7), 801-808.
2. Adamopoulos, D., Argacha, J., Gujic, M., Preumont, N., Degaute, J., & van de Borne, P. (2009). acute effects of nicotine on arterial stiffness and wave reflection in healthy young non-smokers. *Clinical and Experimental Pharmacology and Physiology, 36*(8), 784-789.
3. Ambrose, J., & Barua, R. (2004). The pathophysiology of cigarette smoking and cardiovascular disease - an update. *Journal of the American College of Cardiology, 43*(10), 1731-1737.
4. Arulanantham, K., & Genel, M. (1978). Central nervous system toxicity associated with ingestion of propylene glycol. *The Journal of Pediatrics, 93*(3), 515-516.
5. Benowitz, N. L. (2010). Nicotine addiction. *The New England Journal of Medicine*, 362(24), 2295-2303.
6. Benowitz, N. L., & Burbank, A. D. (2016). Cardiovascular toxicity of nicotine: Implications for electronic cigarette use. *Trends in Cardiovascular Medicine*, 26(6), 515-523.
7. Carnevale, R., Sciarretta, S., Violi, F., Nocella, C., Loffredo, L., Perri, L., Peruzzi, M., Marullo, A.G.M., De Falco, E., Chimenti, I., Valenti, V., Biondi-Zoccai, G., and Frati, G. (2016). Acute impact of tobacco vs electronic cigarette smoking on oxidative stress and vascular function. *Chest, 150*(3), 606-612.

8. Cheng, T. (2014). Chemical evaluation of electronic cigarettes. *Tobacco Control*, 23(suppl 2), pp.ii11-ii17.
9. Crippa, G., Bergonzi, M., Bravi, E., Balordi, V., & Cassi, A. (2018). effect of electronic cigarette smoking on blood pressure in hypertensive patients. evaluation by non-invasive continuous ambulatory blood pressure measurement. *Journal of Hypertension, 36*, e4.
10. Cryer, P. E., Haymond, M. W., Santiago, J. V., & Shah, S. D. (1976). Norepinephrine and epinephrine release and adrenergic mediation of smoking-associated hemodynamic and metabolic events. *The New England Journal of Medicine*, 295(11), 573-577.
11. Czernin, J., & Waldherr, C. (2003). Cigarette smoking and coronary blood flow. *Progress in Cardiovascular Diseases*, 45(5), 395-404.
12. Dajas-Bailador, F., & Wonnacott, S. (2004). Nicotinic acetylcholine receptors and the regulation of neuronal signalling. *Trends in Pharmacological Sciences, 25*(6), 317-324.
13. Daynard, R. (2018). Public health consequences of e-cigarettes: a consensus study report of the National Academies of Sciences, Engineering, and Medicine. *Journal Of Public Health Policy*, *39*(3), 379-381.
14. Endemann, D., & Schiffrin, E. (2004). Endothelial dysfunction. *Journal of the American Society of Nephrology*, 15(8), 1983-1992.
15. Fetterman, J. L., Weisbrod, R. M., Feng, B., Bastin, R., Tuttle, S. T., Holbrook, M., Baker, G., Robertson, R.M., Conklin, D.J., Bhatnagar, A., Hamburg, N. M.

(2018). Flavorings in tobacco products induce endothelial cell dysfunction. *Arteriosclerosis, Thrombosis, and Vascular Biology, 38*(7), 1607-1615.

16. Franzen, K. F., Willig, J., Cayo Talavera, S., Meusel, M., Sayk, F., Reppel, M., Dalhoff, K., Mortensen, Kai., Droemann, D. (2018). E-cigarettes and cigarettes worsen peripheral and central hemodynamics as well as arterial stiffness: A randomized, double-blinded pilot study. *Vascular Medicine*, 1358863X18779694.
17. Gaworski, C. L., Dozier, M. M., Gerhart, J. M., Rajendran, N., Brennecke, L. H., Aranyi, C., & Heck, J. D. (1997). 13-week inhalation toxicity study of menthol cigarette smoke. *Food and Chemical Toxicology, 35*(7), 683-692.
18. Glasser, A. M., Katz, L., Pearson, J. L., Abudayyeh, H., Niaura, R. S., Abrams, D. B., & Villanti, A. C. (2017). Overview of Electronic Nicotine Delivery Systems: A Systematic Review. *American Journal of Preventive Medicine*, *52*(2), e33–e66.
19. Goel, R., Durand, E., Trushin, N., Prokopczyk, B., Foulds, J., Elias, R. J., & Richie, J., John P. (2015). Highly reactive free radicals in electronic cigarette aerosols. *Chemical Research in Toxicology, 28*(9), 1675-1677.
20. Goniewicz, M., Knysak, J., Gawron, M., Kosmider, L., Sobczak, A., Kurek, J., Prokopowicz, A., Jablonska-Czapla, M., Rosik-Dulewska, C., Havel, C., Jacob, P. and Benowitz, N. (2014). Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. *Tobacco Control*, 23(2), 133-139.
21. Grana, R., Benowitz, N., & Glantz, S. A. (2014). E-Cigarettes: A Scientific Review. *Circulation*, *129*(19), 1972–1986.

22. Harris, R. A., Nishiyama, S. K., Wray, D. W., & Richardson, R. S. (2010). Ultrasound assessment of flow-mediated dilation. *Hypertension, 55*(5), 1075-1085.

23. Herrington, J. S., & Myers, C. (2015). Electronic cigarette solutions and resultant aerosol profiles. *Journal of Chromatography A, 1418*, 192-199.

24. Huang, S., Xu, Y. and Lau, A. (2018). Electronic cigarette: A recent update of its toxic effects on humans. Journal of Cellular Physiology, 233(6), pp.4466-4478.

25. Jamal A, Gentzke A, Hu SS, et al. Tobacco Use Among Middle and High School Students — United States, 2011–2016. *MMWR Morbidity And Mortality Weekly Report* 2017; 66:597–603.

26. Javed, F., Kellesarian, S., Sundar, I., Romanos, G. and Rahman, I. (2017). Recent updates on electronic cigarette aerosol and inhaled nicotine effects on periodontal and pulmonary tissues. *Oral Diseases*, 23(8), pp.1052-1057.

27. Jensen, K., Nizamutdinov, D., Guerrier, M., Afroze, S., Dostal, D., & Glaser, S. (2012). General mechanisms of nicotine-induced fibrogenesis. *FASEB Journal, 26*(12), 4778-4787.

28. Johnson CD, Melanaphy D, Purse A, Stokesberry SA, Dickson P, Zholos AV 2009. Transient receptor potential melastatin 8 channel involvement in the regulation of vascular tone. *American Journal of Physiology-Heart and Circulation Physiology 296*: 1868-1877.

29. Kienhuis, A., Soeteman-Hernandez, L., Bos, P., Cremers, H., Klerx, W., & Talhout, R. (2015). Potential harmful health effects of inhaling nicotine-free

shisha-pen vapor: A chemical risk assessment of the main components propylene glycol and glycerol. *Tobacco Induced Diseases, 13*(1), 15.

30. Kubozono, T., Miyata, M., Ueyama, K., Nagaki, A., Otsuji, Y., Kusano, K., Kubozono, O. and Tei, C. (2007). Clinical Significance and Reproducibility of New Arterial Distensibility Index. *Circulation Journal*, 71(1), pp.89-94.

31. Kubozono, T., Miyata, M., Ueyama, K., Hamasaki, S., Kusano, K., Kubozono, O., & Tei, C. (2011). Acute and chronic effects of smoking on arterial stiffness. *Circulation Journal, 75*(3), 698-702.

32. Leigh, N., Lawton, R., Hershberger, P., & Goniewicz, M. (2016). Flavourings significantly affect inhalation toxicity of aerosol generated from electronic nicotine delivery systems (ENDS). *Tobacco Control, 25*(Suppl 2), ii81-ii87.

33. Lin, A., Liu, M., Ko, H., Perng, D., Lee, T., & Kou, Y. (2017). Inflammatory effects of menthol vs. non-menthol cigarette smoke extract on human lung epithelial cells: A double-hit on TRPM8 by reactive oxygen species and menthol. *Frontiers in Physiology, 8*, 263. doi:10.3389/fphys.2017.00263

34. Longo, D. L., Dinakar, C., & O'Connor, G., T. (2016). The health effects of electronic cigarettes. *The New England Journal of Medicine, 375*(14), 1372-1381.

35. Luttrell, W. E., & Vogel, H. F. (2014). Nicotine. *Journal of Chemical Health & Safety*, 21(4), 39-41.

36. Martin, G., & Finberg, L. (1970). Propylene glycol: A potentially toxic vehicle in liquid dosage form. *The Journal of Pediatrics, 77*(5), 877-878.

37. Olfert, I., DeVallance, E., Hoskinson, H., Branyan, K., Clayton, S., Pitzer, C., Sullivan, D.P., Breit, M.J., Wu, Z., Klinkhachorn, P., Mandler, W.K., Erdreich, B.H., Ducatman, B.S., Bryner, R.W., Dasgupta, P., Chantler, P. (2018). Chronic exposure to electronic cigarettes results in impaired cardiovascular function in mice. *Journal of Applied Physiology, 124*(3), 573-582.

38. Youth, E-Cigarette Use Among, and US Department of Health and Human Services. A report of the Surgeon General—executive summary. Atlanta (GA): US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; 2016.

39. Park, W., Miyachi, M., & Tanaka, H. (2014). Does Aerobic Exercise Mitigate the Effects of Cigarette Smoking on Arterial Stiffness? *The Journal of Clinical Hypertension*, *16*(9), 640-644.

40. Pickworth, W. B., Moolchan, E. T., Berlin, I., & Murty, R. (2002). Sensory and physiologic effects of menthol and nonmenthol cigarettes with differing nicotine delivery. *Pharmacology, Biochemistry and Behavior, 71*(1), 55-61.

41. *QuickStats*: Percentage of Adults Who Ever Used an E-cigarette† and Percentage Who Currently Use E-cigarettes,§ by Age Group — National Health Interview Survey, United States, 2016. (2017). *MMWR. Morbidity And Mortality Weekly Report*, *66*(33), 892.

42. Ras, R. T., Streppel, M. T., Draijer, R., & Zock, P. L. (2012;2013;). Flow-mediated dilation and cardiovascular risk prediction: A systematic review with meta-analysis. *International Journal of Cardiology*, 168(1), 344-351.

43. Saiki, A., Sato, Y., Watanabe, R., Watanabe, Y., Imamura, H., Yamaguchi, T., Ban, N., Kawana, H., Nagumo, A., Nagayama, D., Ohira, M., Endo, k., Tatsuno, I. (2016). The role of a novel arterial stiffness parameter, cardio-ankle vascular index (CAVI), as a surrogate marker for cardiovascular diseases. *Journal of Atherosclerosis and Thrombosis*, 23(2), 155-168.

44. Shirai, K., Utino, J., Otsuka, K. and Takata, M. (2006). A Novel Blood Pressure-independent Arterial Wall Stiffness Parameter; Cardio-Ankle Vascular Index (CAVI). *Journal of Atherosclerosis and Thrombosis*, 13(2), pp.101-107.

45. Shirai, K., Hiruta, N., Song, M., Kurosu, T., Suzuki, J., Tomaru, T., Miyashita, Y., Saiki, A., Takahashi, M., Suzuki, K. and Takata, M. (2011). Cardio-Ankle Vascular Index (CAVI) as a Novel Indicator of Arterial Stiffness: Theory, Evidence and Perspectives. *Journal of Atherosclerosis and Thrombosis*, 18(11), pp.924-938.

46. Tölle, M., Reshetnik, A., Schuchardt, M., Höhne, M. and van der Giet, M. (2015). Arteriosclerosis and vascular calcification: causes, clinical assessment and therapy. *European Journal of Clinical Investigation*, 45(9), pp.976-985.

47. Tomiyama, H., Kohro, T., Higashi, Y., Takase, B., Suzuki, T., Ishizu, T., Schinichiro, U., Yamakazi, T., Furumoto, T., Kario, K., Inoue, T., Shinji, K., Watanabe, K., Takemoto, Y., Hano, T., Sata, M., Ishibahi, Y., Node, K.,

Yamashina, A. (2015). Reliability of measurement of endothelial function across multiple institutions and establishment of reference values in Japanese. *Atherosclerosis, 242*(2), 433-442.

48. Tomiyama, H., Saisu, T., & Yamashina, A. (2017). Measurement of flow-mediated vasodilatation: A lesson from the FMDJ study. *International Heart Journal, 58*(2), 158-162.
49. United States. Public Health Service. (2011). *How tobacco smoke causes disease, the biology and behavioral basis for smoking-attributable disease, A report of the surgeon general*, 2010
50. van Berlo, J. H., Maillet, M., & Molkentin, J. D. (2013). Signaling effectors underlying pathologic growth and remodeling of the heart. *The Journal of Clinical Investigation, 123*(1), 37-45.
51. Vlachopoulos, C., Ioakeimidis, N., Abdelrasoul, M., Terentes-Printzios, D., Georgakopoulos, C., Pietri, P., Stephanadis, C., Tousoulis, D. (2016). Electronic cigarette smoking increases aortic stiffness and blood pressure in young smokers. *Journal of the American College of Cardiology, 67*(23), 2802-2803.
52. Varughese, S., Teschke, K., Brauer, M., Chow, Y., van Netten, C., & Kennedy, S. M. (2005). Effects of theatrical smokes and fogs on respiratory health in the entertainment industry. *American Journal of Industrial Medicine, 47*(5), 411-418.
53. Wieslander, G., Norbäck, D., & Lindgren, T. (2001). Experimental exposure to propylene glycol mist in aviation emergency training: Acute ocular and respiratory effects. *Occupational and Environmental Medicine, 58*(10), 649-655.

54. Xiong, S., Wang, B., Lin, S., Zhang, H., Li, Y., Wei, X., Yuanting, C., Wei, X., Zongshi, L., Peng, G., Li,L., Zhigang, Z., Daoyan, L., and Zhu, Z. (2017). Activation of transient receptor potential melastatin subtype 8 attenuates Cold-Induced hypertension through ameliorating vascular mitochondrial dysfunction. *Journal of the American Heart Association, 6*(8), e005495-n/a.

55. Yan, X. S., & D'Ruiz, C. (2015). Effects of using electronic cigarettes on nicotine delivery and cardiovascular function in comparison with regular cigarettes. *Regulatory Toxicology and Pharmacology, 71*(1), 24-34.